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Cell proliferation inhibitors and apoptosis promoters



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ABSTRACT

Cancer is characterised by uncontrolled proliferation and prolonged cell survival. In some cases, tumour formation is the result from aberrant activity of various cell-cycle regulators leading to chromosome instability or from alteration of the apoptosis pathway. Ovarian cancer is an entity in which cell-cycle alterations are common. P53, a key regulator of checkpoint G1, is frequently altered in high-grade serous ovarian cancer. Targeting cell-cycle regulators will lead to mitotic catastrophe and cell death in these tumours. Promoting apoptosis is another target that is gaining interest in ovarian cancer.

In this review, the most relevant evidence of clinical studies in ovarian cancer with compounds targeting cell cycle or promoting apoptosis is summarised.

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1. Introduction

Cells are usually in a state of cell-cycle arrest. In fact, most cells remain in a G0 phase, which can be either transient (quiescent) or permanent (upon terminal differentiation or senescence). Quiescent cells can be triggered to re-enter the cell cycle through stimulation with mitogenic factors. These factors activate cascades of intracellular signalling that lead cyclin-dependent kinases (CDK) 4 and CDK6 to drive progression from G0/G1 into S phase, in which DNA replication will occur [1].

Any damage in DNA occurred during the replication process must be repaired. Two checkpoints allow halting cell-cycle progression in response to DNA damage. If damage is important and cannot be repaired, programmed cell death pathway will be activated leading to a cellular suicide.

Cancer is characterised by uncontrolled proliferation and prolonged cell survival. In some cases, tumour development is the result from aberrant activity of various cell-cycle

regulators leading to chromosome instability or as a consequence of an aberrant apoptosis pathway.

Ovarian cancer is an entity in which cell-cycle alterations are common. Thus cell cycle and apoptosis regulation are interesting targets for anti-cancer therapy in this setting.

2. Methods

A literature search using Medline-Pubmed search engine has been performed. A search was performed including the terms 'ovarian cancer and apoptosis' and 'ovarian cancer and cell cycle'. Only those papers published in the last 5 years related to clinical trials and in English language were selected.

3. Modulators of the cell cycle

In response to DNA damage, cell cycle can be halted in checkpoints, thereby allowing time for DNA repair. The most

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relevant checkpoints are G1/S and G2/M. Depending on the type of DNA damage, ATR or ATM protein kinases phosphorylate and activate checkpoint kinase 1 (CHK1) or CHK2. Activated CHK mediates a G2 checkpoint arrest by phosphorylating CDC25 among others. These events inactivate CDC25 and allow WEE1 to arrest the cell cycle.

WEE1 inhibits the action of its direct substrate cyclin-dependent kinase (CDK) 1 by phosphorylation of the Tyr154 residue resulting in cell-cycle arrest and allowing time for DNA repair. In p53 defective tumours, such as high-grade serous ovarian carcinoma (HGSOC), inhibition of Wee-1 kinase has shown to increase cytotoxicity of DNA damaging agents *in vitro* [2].

The role of CHK1 and WEE1 in cancer is controversial. Mutations of CHEK1 locus, the gene coding for CHK1, have been linked to breast and gastric cancer.

WEE1 kinase is over-expressed in several types of cancer and can be considered a potential target in cancer therapy.

3.1. WEE-1 inhibitors

AZD1775 (formerly MK-1775) is a potent ATP competitive small molecule inhibitor of Wee-1 kinase. AZD1775 was firstly evaluated in a phase I trial with two parts; in part 1, patients received treatment in monotherapy, and in part 2, AZD1775 was administered in combination with gemcitabine, cisplatin or carboplatin. 202 patients were included. The maximum-tolerated and biological effective dosages were established for each combination. pCDK1 was the pharmacodynamic biomarker, and a 50% reduction of pCDK1 in surrogate tissue was observed in combination with carboplatin and cisplatin. The response rate in mutp53 cancer patients (N = 19) was 21% compared with 12% in p53 wild-type patients (N = 33). These results suggested the potential efficacy of AZD1775 in combination with platin salts in TP53-mutated tumours.

Therefore, in a phase II trial [3], the combination of AZD1775 225 mg twice a day over 2.5 days every 21 days and carboplatin AUC 5 in TP53 mutated ovarian cancer refractory or resistant to first-line chemotherapy (CT) was assessed. Of note, the definition of platinum resistance criteria in this trial was relapse after a platinum-free interval <3 months. This combination showed encouraging activity in a poor-prognosis population. 23 patients, of which 39% were primary refractory to first line, were included. Most patients had serous histology (70%) and only 9% harboured BRCA mutations. Of note, TP53 mutations were analysed by both IHC and sequencing in archived samples.

The overall response rate in this poorly responsive population was 43% (95% confidence interval (CI) 22–66%). The median progression-free survival (PFS) was 5.3 months (95% CI 2.3–9.0 months) and median overall survival (OS) was 12.6 months (95%CI 4.9–19.7 months) providing clinical proof of the activity of this strategy. The toxicity of the combination of AZD1775 and carboplatin was manageable, with fatigue (87%), nausea (78%) and thrombocytopenia (70%) being the most frequent toxic effects. The most frequent grade 3 or 4 adverse events were thrombocytopenia (48%) and neutropenia (37%). This study suggests the relevance of inhibiting a cell-cycle regulator in mutp53 ovarian cancer and warrants further development of this strategy.

3.2. CHK inhibitors

Prexasertib (LY2606368) is an ATP-competitive protein kinase inhibitor of checkpoint kinases 1 (CHK1) and CHK2. In the phase I trial [4] with prexasertib, 45 patients were treated at different dose levels. Prexasertib was well tolerated with neutropenia being the most relevant grade 3–4 adverse event. In fact, grade 4 neutropenia occurred in 73.3% of patients but was transient (<5 days). The two patients achieving a partial response had squamous cell carcinomas. The final recommended dose for phase II was 105 mg/m² once every 14 days.

In an initial communication of a phase II trial [5], data from 22 patients with recurrent heavily pre-treated ovarian cancer was reported. Patients treated with prexasertib monotherapy were included in two cohorts according to gBRCA mutations: cohort 1 with gBRCA wild-type patients and cohort 2 with gBRCA mutated. In cohort 1, prexasertib achieved 38% overall response rate (ORR) in 13 evaluable patients. However, in gBRCA-mutated patients, no responses were seen in the six evaluable patients, although two patients achieved stable disease for >4 months. Haematologic toxicity was the most relevant adverse event. Although limited by small sample size, the ORR in this population was encouraging.

More recently, the results from a two-stage phase II trial in a gBRCA wild-type cohort have been published [6]. In this study, 28 ovarian cancer patients were included. Most patients (79%) had platinum-resistant or platinum-refractory disease. All women received at least one dose of prexasertib, but 14% were not assessable for RECIST response. Eight (33%) of 24 patients had partial response. More common grade 3–4 adverse events were neutropenia (93%) and thrombocytopenia (25%).

4. Targeting apoptosis

Apoptosis (type 1 programmed cell death) is a mechanism that eliminates abnormal cells that pose a serious threat to the organism's life. Thus, apoptosis is one of the most relevant mechanisms of tumour control. Occasionally, a cell can acquire mutations that allow it to avoid apoptotic death, thus enabling malignant progression [7]. The abnormal expression of these anti-apoptotic molecules can make anti-cancer therapies less effective. Therefore activation of programmed cancer cell death is a promising strategy to overcome cancer resistance.

4.1. Inhibitor of apoptosis proteins (IAP) modulators

The IAP proteins are a family of eight human proteins that function as endogenous inhibitors of caspases, the proteins that lead to apoptosis. However, besides regulating apoptosis, IAP proteins have also been implicated in the control of non-apoptotic processes including differentiation, migration, invasion and metastasis. IAP also regulates nuclear factor kappa beta (NF- κ B) signalling [8].

Debio-1143 is a potent orally active IAP antagonist that promotes apoptosis in tumour cells by restoring caspase activity. Moreover, Debio-1143 is also a second mitochondria-derived activator of caspase (SMAC) mimetic. Debio-1143 has

shown a synergistic effect with taxanes and topoisomerase inhibitors [9]. This compound is currently under evaluation in combination with carboplatin and paclitaxel in the neoadjuvant setting of newly diagnosed ovarian cancer patients in a randomised phase II trial versus placebo (EUDRA-CT-2015-005137-42).

Birinapant (TL32711), a SMAC mimetic that efficiently degrades IAP, has shown to overcome platinum resistance in ovarian cancer cell lines in combination with carboplatin [10]. This compound has been tested in a phase II clinical trial in ovarian cancer (NCT01681368).

4.2. Other; lurbinectedin (PM01183) is a tetrahydropyrrolo-quinoline alkaloid analogue that inhibits RNA polymerase II activity and regulates the micro-environment

Pre-clinical experiments have shown that lurbinectedin has an impact on the apoptosis regulation by increasing the apoptosis mediated by CK-18 and dependent on caspase activity [11].

A phase II clinical trial [12] assessed the activity of lurbinectedin in platinum-resistant or refractory ovarian cancer. In a first stage (N = 22), the activity of lurbinectedin single agent at 7.0 mg flat dose every 21 weeks was confirmed, and in a second stage (N = 59) patients were randomised to receive lurbinectedin versus topotecan. The primary end-point was overall response rate (ORR) by RECIST and/or GCIG criteria. ORR of all lurbinectedin-treated patients (N = 52) was 23% (95%CI, 13%–37%). The highest activity of lurbinectedin was seen in platinum-resistant disease (ORR = 30%). No responses in the arm of topotecan were seen. Grade 3–4 neutropenia in 85% of patients was the most relevant toxicity of lurbinectedin.

In ESMO 2018, the results of the CORAIL study (NCT02421588) were presented [13]. This was a phase III trial comparing lurbinectedin and pegylated liposomal doxorubicin (PLD) in platinum-resistant ovarian cancer patients treated with no more than three prior lines. The primary end-point was progression-free survival (PFS), and the study was powered to demonstrate a 30% reduction in the relative risk of progression or death. 442 patients were randomised. PFS was 3.5 months in the lurbinectedin arm versus 3.6 in the PLD/topotecan arm. The study did not meet its primary end-point of 30% reduction in PFS.

5. Targeting p53: induction of cell-cycle arrest and apoptosis

The tumour-suppressor p53 (TP53) is widely mutated in cancer [14], including in over 96% of HGSOV. Mutations cause loss of wild-type p53 function. In the absence of cellular stress, wild-type p53 is maintained at low levels by ubiquitin ligase MDM2 that ubiquitinates p53 marking it for proteasomal degradation. In response to stress, numerous mechanisms act to disrupt MDM2-p53 association resulting in stabilisation and activation of p53. Activated p53 promotes processes consistent with tumour suppression, including cell-cycle inhibition, apoptosis, senescence, DNA repair and autophagy.

Some studies suggest that p53 immunohistochemistry (IHC) may be used as a surrogate marker of TP53 mutations [15]. Thus, p53 detection by IHC has been used as a potential biomarker for patient selection in trials with compounds targeting mutp53.

However, there is growing evidence that mutant p53 (mutp53) results in both loss of p53 wild-type oncosuppressive activity and gain functions that help to contribute to malignant progression [16].

There are several strategies that target p53 in different tumour types. The two most developed compounds in ovarian cancer are:

PRIMA-1 (p53 re-activation and induction of massive apoptosis) also named APR-017 and the more active methylated derivative APR-246 (PRIMA-1-MET) are a new family of compounds with potential antineoplastic activity [17]. APR-246 modifies the core domain of the mutant forms of p53 by alkylation of thiol groups. These modifications have shown to restore p53 wild-type endogenous activity leading to cell-cycle arrest and apoptosis.

This pharmacological restoration of p53 by APR-246 is being evaluated in the clinics.

In a phase Ib study of APR-246 in combination with carboplatin and pegylated liposomal doxorubicin in HGSOV in platinum-sensitive relapse, the main toxicity attributed to APR-246 was dizziness, which occurred in 71% of patients (mainly grade 1–2). Signs of activity were seen, mainly in the high-doses cohort in which ORR was 88% (7/8) with three complete responses [18].

Two phase II trials with APR-246 combined with chemotherapy in p53 mutant ovarian cancer patients in both platinum-sensitive (P53 suppressor activation in recurrent high-grade serous ovarian cancer (PiSARRO) trial, NCT02098343) and in platinum-resistant relapse (PiSARRO-R trial, NCT03268382) are ongoing.

Ganetespib is an inhibitor of the heat shock protein 90 (HSP90) core protein.

The HSP90 chaperone machinery is highly activated in cancers compared to normal tissues and renders them resistant to proteotoxic stress by supporting proper folding of conformationally aberrant oncoproteins including mutp53 [19]. In this context the inhibition of HSP90 mediates destabilisation and degradation of mutp53. Ganetespib has been evaluated in combination with weekly paclitaxel in p53 mutant platinum-resistant ovarian cancer in the GANNET53 trial [20]. This trial was prematurely closed for active recruitment due to unsecured drug supply of ganetespib. 133 patients (of the foreseen 222) were included. The addition of ganetespib to paclitaxel showed no improvement in survival. Median PFS was 3.5 and 5.3 months for paclitaxel + ganetespib and ganetespib single agent, respectively. The most frequent side effect for the combination was diarrhoea (79% had grade 1–2).

Ganetespib is currently being investigated in combination with either carboplatin or niraparib in the EUDARIO trial (NCT03783949). This is a multi-centre, open-label three-arm phase II trial in platinum-sensitive ovarian cancer patients. Estimated enrolment is 120 patients and results are awaited for 2022.

6. Conclusions

While basic cell-cycle regulators were discovered decades ago, in the last years our understanding of their role in cancer and as potential targets for cancer therapy has experienced a dramatic increase. The provisional approval by the FDA of the CDK4/CDK6 inhibitor palbociclib in breast cancer represents a first successful clinical translation in this field.

New developed compounds targeting p53 are increasing the therapeutic armamentarium. However, a number of hurdles still need to be overcome before the studies in mutp53 ovarian cancer patients can be translated into clinical practice. While there is clear evidence that mutp53 promotes various oncogenic responses, some critical pathways remain unclear. Moreover, how differently mutations affect p53 function is also under-explored.

Finally, pharmacological modulation of apoptosis pathway is a very interesting point in those tumours, as in HGSOC, with p53 mutations that lead to an impaired apoptosis regulation.

Declaration of competing interest

The author states the following conflicts of interest:

- Speaker bureau from AstraZeneca, Pharmamar, Ipsen, Roche and Lilly.
- Travel expenses from Roche, AstraZeneca and Pfizer.
- Advisory boards from clovis, tesaro, clinigen and astraZeneca.

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